A Useful Binary Matrix for Visible-MALDI of Low Molecular Weight Analytes

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In this work, a new absorbing candidate, rhodamine (R) 575, is described, which forms the basis of a binary matrix operating at 532 nm. Analyte ionization is found to be much more efficient when the dye is combined with a proton donor such as hydrochloric acid or α-cyano-4-hydroxycinnamic acid, or a proton acceptor such as sodium hydroxide. This makes the matrix more generic than many others that have been tried. Furthermore, under visible illumination R575 produces very few chemical fragments, making it useful for small molecular weight analyte detection. Spectra for a variety of analytes are shown. Insight into the MALDI mechanism was obtained by comparing the similarities and differences of visible-MALDI with the more common UV and IR-MALDI strategies. (J Am Soc Mass Spectrom 2010, 21, 294–299) © 2010 American Society for Mass Spectrometry

Over the last two decades, matrix assisted laser/desorption ionization (MALDI) mass spectrometry (MS), using either ultraviolet (UV) [1, 2] or infrared (IR) lasers [3], has become an important analytical tool for the analysis of molecules of chemical and biological interest. The success of MALDI-MS is largely due to the relative "softness" of this technique, which allows for ionization and detection of intact analytes with minimal fragmentation. IR-MALDI has been shown to be superior to UV-MALDI in this regard for the analysis of biological macromolecules including DNA and RNA [4], although the former technique typically operates at higher pulse energies and features higher sample consumption per laser pulse. Today, UV lasers and, in particular, the N_2 gas lasers with an output wavelength of 337 nm and Nd:YAG lasers with a wavelength of 355 nm dominate in MALDI-MS instruments; and they are usually less expensive than their IR counterparts.

One could expect that MALDI mass spectrometry using visible wavelength lasers will have several advantages over UV-MALDI and IR-MALDI. Many macromolecules of interest absorb UV-light but are transparent at visible wavelengths. Evidence suggests that visible-MALDI is softer than UV-MALDI [5]. Similar to UV-MALDI, visible-MALDI is expected to require lower pulse energies than IR-MALDI. Furthermore, readily available pulsed visible laser sources such as the solid-state frequency-doubled Nd:YAG laser (532 nm wavelength) are becoming relatively inexpensive and have longer operational lifetimes compared with standard UV devices. Delivery of light via fiber optics is also more routine at visible wavelengths.

Despite its potential, visible MALDI has not been extensively explored. Studies have dealt with rhodamine (R) B, R6G [5, 6], neutral red [7], and 2-amino-3-nitrophenol [8] as possible matrices for visible-MALDI using the 532 nm output of a doubled Nd:YAG laser. Recently, Au-assisted visible laser MALDI has emerged [9] as a new analysis technique as well as visible surface-assisted laser desorption/ionization from a graphite substrate [10]. Our group recently studied visible-MALDI coumarin laser dyes as potential MALDI matrixes using the 480 nm output of an optical parametric oscillator [11]. While all these compounds performed adequately, they were, for the most part, characterized by a high degree of background chemical noise. It is our contention that visible-MALDI will only be widely accepted if a matrix can be found that is comparable in performance to standard UV compounds, such as CHCA and DHB, and/or exhibits low chemical noise. It is also important that visible-MALDI be carried out using standard, reliable, and increasingly inexpensive devices such as the doubled output of a Nd:YAG laser at 532 nm.

The key problem that has not been addressed is the paucity of known organic molecules that can absorb 532 nm laser light and serve as matrixes. Laser dyes are natural candidates as visible MALDI matrices [11] or as the laser absorber in a binary matrix combination [6]. Rhodamine dyes, in particular, strongly absorb 532 nm laser radiation and, as aromatic compounds, are relatively stable towards fragmentation. This work focuses on one particularly promising candidate: the neutral laser dye R575, (2-[6-(ethyl amino)-3-(ethylimino)-2,7-dimethyl-3H-xanthen-9-yl]-benzoic acid) whose zwitte-

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1044-0305/10/$32.00
doi:10.1016/j.jasms.2009.10.016

Published online October 29, 2009
Received July 17, 2009
Revised October 22, 2009
Accepted October 22, 2009

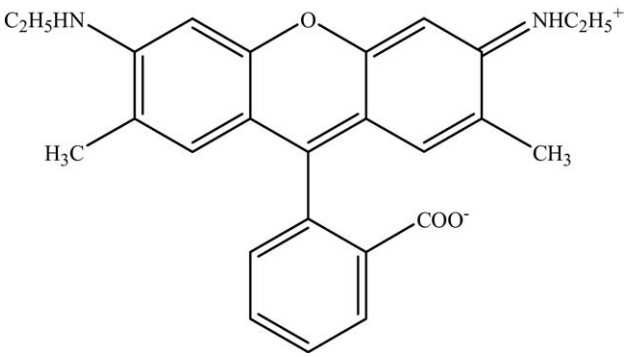

Figure 1. Chemical structure of R575.

rionic structure is shown in Figure 1 [12]. When R575 dye is mixed with an acid or base, analyte ionization can be achieved by either addition or abstraction of the proton, respectively. Although a two-component mixture is a bit more complex, the binary matrix becomes more generic and flexible.

Experimental

The experimental arrangement used to record MALDI mass spectra has been reported elsewhere [13]. Briefly, the instrument is based on Applied Biosystems/MDS SCIEX API-365 LC/MS/MS triple quadrupole (QqQ) mass spectrometer originally configured for electrospray ionization (ESI) and now equipped with a home-made MALDI ion source, which has been described in reference [14]. The spectra obtained from our MALDI mass spectrometer using UV (337 nm irradiation) were compared with those obtained using a commercial instrument (Bruker Reflex IV). The latter device disperses ions by time-of-flight (TOF). It was found that under similar fluences (but different energies and spot sizes) the sensitivity of the home-made MALDI source is typically ~one order of magnitude less than that of the commercial MALDI-TOF instrument.

Each overview spectrum, averaged over multiple laser shots, was obtained by scanning the first quadrupole (Q_1) across the mass range of 50 to ~2200 Da. To reduce data acquisition times, shorter scans could be taken over the mass region of the analyte of interest such as m/z = 720 to 730 for dalargin, whose mass signal comes at m/z = 726.4. Although MALDI TOF is the most common approach used for biomolecules, quadrupole instruments are now finding great utility for the detection of low molecular weight analytes [14–16]. The Q_0 quadrupole facilitated collection and collisional focusing of MALDI ions. Spectra were recorded in either positive or negative ion mode.

Scanning Electron Microscope (SEM) images of the solid matrix samples were obtained with a Hitachi S-4500 field emission microscope (Hitachi High-Technologies Corporation, Tokyo, Japan).

Five hundred thirty-two nm photons were generated as the second-harmonic of a Q-switched Nd:YAG laser operating at 1064 nm (Quanta-Ray GCR-3). The pulse width and repetition rate were ~4 ns and 10 Hz, respectively. The laser beam was introduced into a 100 μm diameter optical fiber using a 5 cm focal length lens and focused onto the sample surface (area ~0.01 mm^2). The typical laser energy emerging from the output of the optical fiber was ~17 uJ per pulse, and was set by adjusting a half-wave plate and polarizer combination and measuring with a power meter (Ophir Electronics, Jerusalem, Israel, model NOVA). The position of the laser beam was fixed while the sample plate was rotated, resulting in an observable ablation ring on the sample surface. The area covered by the ablation ring was measured and used to calculate the average amount of analyte consumed by the laser. For each quantitative measurement, the sample within the ablation ring was completely consumed.

A 2 mM solution of R575 (Exciton, Dayton, OH, USA, molar mass, MM = 414.49 g, $C_{26}H_{26}N_2O_3$), in ethanol was prepared using the reagents as purchased. The main impurities in the dye to a 1% level of contamination were established by the manufacturer to be NaOH, R6G, and RB. The UV-visible spectrum of a typical R575 ethanol solution is shown in Figure 2a, while the solid-state spectrum is presented in Figure 2b. As expected, the solid-state spectrum is broader than the solution spectrum but the absorption maxima are similar. The solid-state spectrum of a sample with the optimum amount of HCl added shown in Figure 2c is very similar to that in Figure 2b, although the peak maximum is slightly red-shifted.

Our selection of organic analytes for evaluation of visible-MALDI was limited to those with parent ion molecular weights within the detection mass range of our quadrupole mass analyzer. The compounds chosen were all small drugs and peptides with masses that are

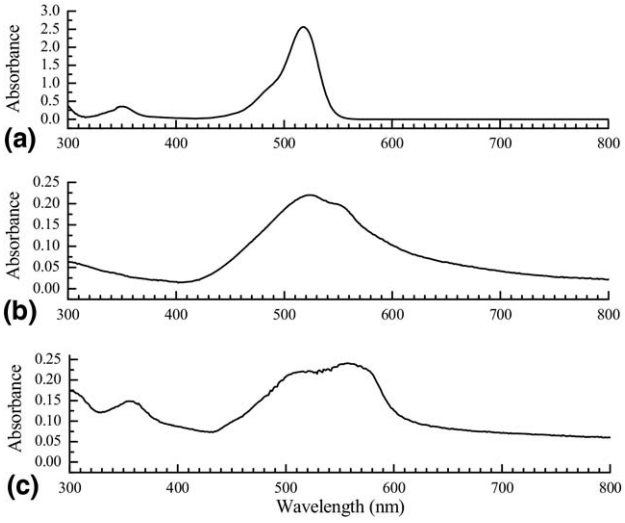

Figure 2. (**a**) UV-visible absorption spectrum of R575 in ethanol; (**b**) UV-visible absorption spectrum of a solid film of R575; (**c**) UV-visible spectrum of a solid R575/HCl solution where the ratio of dye to acid is 2:1.

often overlapped with matrix peaks in UV-MALDI. These species included Bradykinin (MM = 1060.21 g mol^{-1}, $C_{50}H_{73}N_{15}O_{11}$), a polypeptide containing nine amino acid residues, dalargin (MM = 725.8 g mol^{-1}, $C_{35}H_{51}N_9O_8$), a polypeptide containing six amino acid residues, and the pesticide bentazone (MM = 240.28 g mol^{-1}, $C_{10}H_{12}N_2O_3S$). dalargin has both a basic (pKa = 13.55 ± 0.70) group and acidic carboxylic acid functional group (pKa = 3.55 ± 0.21) [17] located on the Arg residue. This peptide also has a high sodium affinity. Bentazone is acidic, with a pKa = 3.3 [18]. The water solubility of dalargin and bradykinin is 1 mg/mL, but were dissolved in a mixture of acetonitrile and deionized water to increase the rate of solvent evaporation. A solution volume ratio of acetonitrile:water = 30:70 and 70:30 were used for dalargin and bradykinin, respectively. Bentazone was dissolved in neat ethanol.

Matrix samples were prepared in this work using the "Dried-Droplet Method" [2, 19]. The matrix and analyte solutions were premixed in a small plastic vial, and then spotted onto a sample probe tip. The mixture was quickly dried by heating using an air heater before use.

Results and Discussion

A variety of experiments were carried out to characterize the utility of R575 as a matrix for visible-MALDI.

The Matrix Under Visible Illumination

The dominant ion from a freshly prepared R575 under 532 nm irradiation without any co-deposited analyte in positive ion mode is protonated R575:$[R575 + H]^+$ plus a few readily identifiable chemical fragments and adducts. Very weak signals were also detected in negative ion mode.

R575 as a Laser Absorber in a Binary Matrix

Protonated dalargin (Dal) signals, $[Dal + H]^+$, were very weak when R575 and Dal were co-deposited in a molar ratio of 1000:1 as the matrix and analyte, respectively (Figure 3d). Under acidic conditions R575 is expected to be a weak conjugate acid: R = $NHC_2H_5^+$, with proton-donating capability. As shown in Figure 2, R575 efficiently absorbs light at 532 nm. It also absorbs efficiently at this wavelength under acidic and basic conditions. Thus, we were able to study enhancement of the analyte signal due to the addition of different acidic and basic compounds to the R575 matrix.

The proton donors tested were hydrochloric acid (HCl, pKa = −7), trifluoroacetic acid (TFA, pKa = 0.52) [20] and α-cyano-4-hydroxycinnamic acid (CHCA, pKa = 1.17 ± 0.31) [21]. These compounds when combined with R575 did not create additional chemical fragments in positive ion mode, or inhibit the formation of $[R575 + Na]^+$ and $[R575 + K]^+$. As can be seen in Figure 3, HCl was found to be the most effective in promoting ionization of dalargin (both overview spectrum and inset Figure 3a), followed by CHCA (inset Figure 3b) and TFA (inset Figure 3c). Furthermore, HCl increases the solubility of R575 in ethanol. Visible-MALDI spectra of Bradykinin recorded in positive ion mode were found to be similar to those obtained for dalargin.

To find the optimal recipe for the R575:HCl binary matrix, a series of spectra containing fixed amounts of

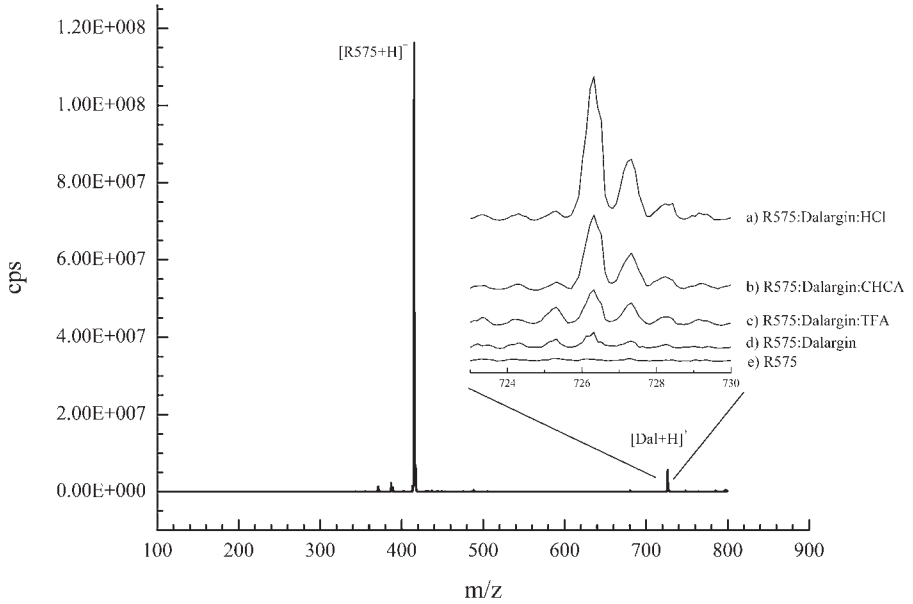

Figure 3. Overview visible MALDI spectrum of dalargin:R575:HCl = 1:12:12. The inset is MALDI mass spectra of (**a**) R575:HCl:dalargin molar ratio = 1000:500:1; (**b**) R575:CHCA:dalargin molar ratio = 1000:508:1; (**c**) R575:TFA:dalargin molar ratio = 1000:508:1; (**d**) R575:dalargin molar ratio = 1000:1; and (**e**) R575 only, in the mass region.

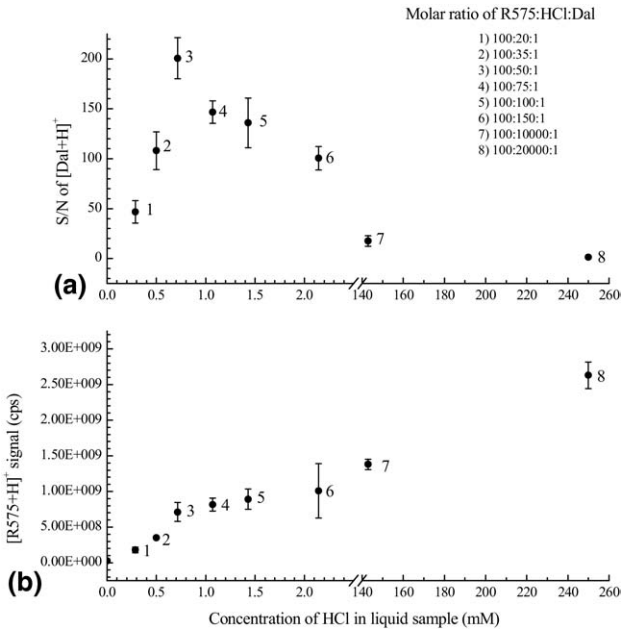

Figure 4. (**a**) A plot of the signal-to-noise ration (S/N) of the protonated dalargin signal as a function of HCl concentration; (**b**) A plot of the protonated matrix signal as a function of HCl concentration.

R575 and dalargin but different amounts of HCl were recorded. As shown in Figure 4a, the S/N of the $[Dal + H]^+$ signal was maximized when the R575:HCl:Dal molar ratio was 100:50:1. However, as shown in Figure 4b, the signal of $[R575 + H]^+$ increased almost linearly with increasing HCl concentration.

To understand this effect, SEM images of the matrix were taken to probe the effect of acid on the crystallization of the matrix. As shown in Figure 5a, the film made with an optimum amount of HCl is relatively smooth while those made with TFA (Figure 5b) and CHCA (Figure 5c) are relatively patchy. This suggests that the analyte ion signal dependence on acid concentration reflects in large part the homogeneity of the matrix sample; that is, there is less dependence on "sweet spots" when using HCl.

Detection Sensitivity

The detection limit for dalargin of $(2 \pm 1) \times 10^{-13}$ mol was obtained by extrapolating a plot of signal-to-noise ratio (S/N) versus absolute analyte concentration obtained from a series of samples where the amount of R575 and HCl (molar ratio = 2:1) was held constant but the analyte concentration was changed. A similar detection limit was found for bradykinin.

The mass peak used to obtain the S/N ratio was that of $[Dal + H]^+$ at m/z = 726.44, while the noise was determined by measuring the background signal in the mass range between m/z = 720 and 724. A value of S/N = 5 was considered as a minimum value that would allow the peak signal to be clearly differentiated from the noise.

Since the signal of $[R575 + H]^+$ could be enhanced by the addition of a proton donor, it was anticipated that R575 would be a good matrix for detecting acidic analytes in negative ion mode. Indeed, deprotonated bentazone could be easily detected in negative ion mode using only R575 as the matrix.

The intensity of the deprotonated bentazone peak was dramatically increased by the addition of NaOH into the matrix. A molar ratio of R575:NaOH:bentazone = 1200:10:1 produced a spectrum with the best signal-to-noise ratio. In this way, sample amounts as small as 12 fmol could be detected.

We think that formation of deprotonated bentazone ion is enhanced by the addition of NaOH because R575 has a higher affinity for sodium ions rather than protons. Bentazone is acidic and therefore will react with NaOH to form sodium bentazone [22] and H_2O. R575 is then sodiated to $[R575 + Na]^+$, thereby enhancing the $[Ben - H]^-$ signal. However, when excessive amount of NaOH was used, the $[Ben - H]^-$ signal disappeared, due to suppression of the interaction between R575 and sodium bentazone.

We believe that better detection limits for each analyte could be obtained on a modern MALDI-TOF mass spectrometer configured to operate at the 532 nm wavelengths due to better efficiency of ion transmission.

Influence of Laser Fluence on Ion Yield

The effect of laser fluence on analyte signal intensity was studied to gain insight into visible-MALDI process and make a comparison to UV- and IR-MALDI techniques [23, 24]. In our experiments, we followed the methodology reported previously [11]. Signal intensities for dalargin MH^+ and R575 MH^+ ions versus laser fluence are presented in Figure 6 as log–log plots. In this

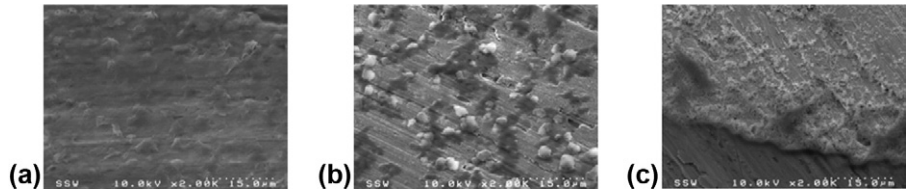

Figure 5. SEM images of R575 + acid + dalargin solid films. (**a**) molar ratio of R575:HCl:Dal = 1000:500:1; (**b**) molar ratio of R575:TFA:Dal = 1000:508:1; (**c**) molar ratio of R575:CHCA:Dal = 1000:537:1. The scale bar shown on each figure corresponds to 15.0 ìm.

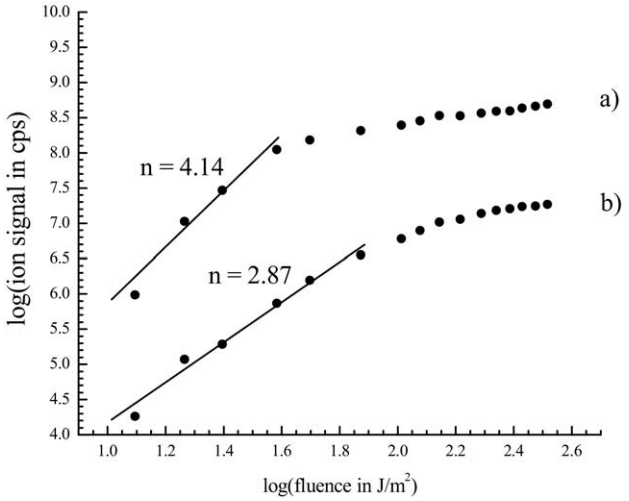

Figure 6. Log-log plot of the ion signals of (**a**) $[R575 + H]^+$ and (**b**) $[Dal + H]^+$ versus laser fluence from 12 ~ 269 J/m^2 at 532 nm.

study, we used the optimal binary matrix with 2:1 R575 to HCl ratio. The slopes found for the matrix and analyte ions are ~4 and 3, respectively. These slopes are lower than those found for coumarin dyes [11] as a matrix for visible MALDI and for conventional matrixes under UV and IR illumination [23, 24]. Thermal desorption and ionization [25] is highly dependent on laser fluence [23, 24]. A lower slope probably means that ionization is less dependent on the availability of proton donors, which in other cases are likely activated via a thermal mechanism. A second factor that may be at play here is rapid radiative relaxation of R575 after it is pumped to its S_1 excited-state [26] by the 532 nm laser irradiation. Finally, a smaller slope may arise because the primary absorption step is two-photon in nature. Thus, more energy is required to achieve sufficient heating of the matrix for desorption and ionization.

Conclusions

We demonstrate that R575 dye in combination with acids such as HCl or CHCA, or bases such as NaOH forms efficient binary matrixes for visible-MALDI at the wavelength of 532 nm. This dye matrix can be used to detect small molecular weight basic and acidic compounds in positive and negative ion modes, respectively. This work was carried out using a triple quadrupole mass spectrometer. However, the results show that a binary matrix of R575 + HCl would be very effective using time-of-flight techniques due to the lack of chemical noise and fragments generated by 532 nm irradiation.

The basic structural framework of the rhodamine dyes is common, although the substituents around the rings shift the absorption profile into different regions in the red portion of the spectrum. Like DHB [27], where small isomeric changes have a dramatic effect on the efficacy of its behavior as a MALDI matrix, a similar effect is observed here. Clearly, not every rhodamine dye behaves the same as a matrix. R575 appears to be the cleanest matrix component discovered to date for visible-MALDI.

The main advantage of using R575 is that it efficiently absorbs 532 nm light, and can serve as a component of a binary matrix for visible-MALDI. Experiments were carried out to compare the detection sensitivity of R575 + HCl relative to the UV matrix: CHCA. The UV matrix proved to be ~1 order of magnitude more sensitive than the best visible binary matrix. However, this is not perceived to be a strong negative because the low-weight mass region is very congested when using CHCA compared with R575 + HCl, which is very clean. This means that detecting low molecular weight compounds should in fact be easier using the visible matrix described in this paper.

To the best of our knowledge, the fluence study here for R575 + HCl is the first to be reported for any rhodamine dye. The study here suggests that the MALDI mechanism at low fluences is thermal in nature, similar to the results found for UV and IR-MALDI but that somewhat higher fluences are required perhaps due to efficient radiative relaxation. The upper mass range of analytes that can be ionized using R575 visible-MALDI remains unknown due to the limited mass range of our quadrupole mass spectrometer. Based on our current evaluation, binary mixtures containing R575 produce very few chemical fragments, which makes them attractive for the analysis of small molecular weight organic compounds [14, 15, 16].

Acknowledgments

The authors acknowledge support for this work by the Natural Science and Engineering Research Council of Canada (NSERC), the Ontario Centers of Excellence, MDS Analytical Technologies, and the University of Western Ontario.

References

1. Karas, M.; Bachmann, D.; Hillenkamp, F. Influence of the Wavelength in High-Irradiance Ultraviolet Laser Desorption Mass Spectrometry of Organic Molecules. *Anal. Chem.* **1985,** *57,* 2935–2939.
2. Karas, M.; Hillenkamp, F. Laser Desorption Ionization of Proteins with Molecular Masses Exceeding 10,000 Daltons. *Anal. Chem.* **1988,** *60,* 2299–2301.
3. Berkenkamp, S.; Menzel, C.; Kara, M.; Hillenkamp, F. Performance of Infrared Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry with Lasers Emitting in the 3im Wavelength range. *Rapid Commun. Mass Spectrom.* **1997,** *11,* 1399–1406.
4. Berkenkamp, S.; Kirpekar, F.; Hillenkamp, F. Infrared MALDI Mass Spectrometry of Large Nucleic Acids. *Science* **1998,** *281,* 260–262.
5. Tang, K.; Allman, S. L.; Jones, R. B.; Chen, C. H. Comparison of Rhodamine Dyes as Matrices for Matrix-assisted Laser Desorption/Ionization Mass Spectrometry. *Org. Mass Spectrom.* **1992,** *27,* 1389–1392.
6. Cornett, D. S.; Duncan, M. A.; Amster, I. J. Liquid Mixtures for Matrix-Assisted Laser Desorption. *Anal. Chem.* **1993,** *65,* 2608–2613.
7. Smith, C. J.; Chang, S. Y.; Yeung, E. S. Characterization of Neutral Red as a Visible Wavelength MALDI Matrix. *J. Mass Spectrom.* **1995**, *30,* 1765–1767.
8. Chen, L. C.; Asakawa, D.; Hori, H.; Hiraoka, K. Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Using a Visible Laser. *Rapid Commun. Mass Spectrom.* **2007,** *21,* 4129–4134.
9. Chen, L. C.; Mori, K.; Hori, H.; Hiraoka, K. Au-Assisted Visible Laser MALDI. *Int. J Mass Spectrom.* **2009,** *279,* 41–46.
10. Kim, J.; Pack, K.; Kang, W. Visible Surface-Assisted Laser/Desorption/Ionization Mass Spectrometry of Small Macromolecules Deposited on the Graphite Plate. *Bull. Korean Chem. Soc.* **2002,** *23,* 315–319.

11. Hu, X. K.; Lacey, D.; Li, J.; Yang, C.; Loboda, A. V.; Lipson, R. H. Visible Wavelength MALDI Using Coumarin Laser Dyes. *Int. J. Mass Spectrom.* **2008,** *278,* 69–74.
12. http://www.exciton.com/pdfs/r575.pdf.
13. Lacey, D.; Hu, X. K.; Loboda, A. V.; Mosey, N. J.; Lipson, R. H. Aspirin Revealed a Cationization Strategy for Detecting Acetylsalicylic Acid by MALDI Mass Spectrometry. *Int. J. Mass Spectrom.* **2007,** *261,* 192–198.
14. Corr, J. J.; Kovarik, P.; Schneider, B. B.; Henfrikse, J.; Loboda, A.; Covey, T. R. Design Consideration for High Speed Quantitative Mass Spectrometry with MALDI Ionization. *J. Am. Soc. Mass Spectrom.* **2006,** *17,* 1129–1141.
15. Cohen, L. C.; Gusev, A. I. Small Molecule Analysis by MALDI Mass Spectrometry. *Anal. Bioanal. Chem.* **2002,** *373,* 571–586.
16. Gobey, J.; Cole, M.; Janiszewski, J.; Covey, T.; Chau, T.; Kovarik, P.; Corr, J. Characterization and Performance of MALDI on a Triple Quadrupole Mass Spectrometer for Analysis and Quantification of Small Molecules. *Anal. Chem.* **2005,** *77,* 5643–5654.
17. SciFinder Scholar, Registry Number: 81733-79-1; Property Calculated Using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris 1994–2009 ACD/Labs.
18. Guimont, S.; Perrin-Ganier, C.; Real, B.; Schiavon, M. Effect of Soil Moisture and Treatment Volume on Bentazon Mobility in Soil. *Agron. Sustain. Dev.* **2005,** *25,* 323–329.
19. Vaidyanathan, S.; Winder, C. L.; Wade, S. C.; Bell, D. B.; Goodacre, R. Sample Preparation in Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry of Whole Bacterial Cells and the Detection of High Mass (>20 kDa) Proteins. *Rapid Commun. Mass Spectrom.* **2002,** *16,* 1276–1286.
20. Namazian, M.; Zakery, M.; Noorbala, M. R.; Coote, M. L. Accurate Calculation of the pKa of Trifluoroacetic Acid Using High-Level Ab Initio Calculations. *Chem. Phys. Lett.* **2008,** *451,* 163–168.
21. Schaiberger, A. M.; Moss, J. A. Optimized Sample Preparation for MALDI Mass Spectrometry Analysis of Protected Synthetic Peptides. *J. Am. Soc. Mass Spectrom.* **2008,** *19,* 614–619.
22. http://www.alanwood.net/pesticides/derivatives/bentazone-sodium.html.
23. Dreisewerd, K.; Schürenberg, M.; Karas, M.; Hillenkamp, F. Influence of Laser Intensity and Spot Size on the Desorption of Molecules and Ions in Matrix-Assisted Laser Desorption/Ionization with a Uniform Beam Profile. *Int. J. Mass Spectom. Ion Processes* **1995,** *141,* 127–148.
24. Westmacott, G.; Ens, W.; Hillenkamp, F.; Dreisewerd, K.; Schurenberg, M. The Influence of Laser Fluence on Ion Yield in Matrix-Assisted Laser Desorption Ionization Mass Spectrometry. *Int. J. Mass Spectrom.* **2002,** *221,* 67–81.
25. Zenobi, R.; Knochenmuss, R. Ion Formation in MALDI Mass Spectrometry. *Mass Spectrom. Rev.* **1998,** *17,* 337–366.
26. Knochenmuss, R. A Quantitative Model of Ultraviolet Matrix-Assisted Laser Desorption/Ionization. *J. Mass Spectrom.* **2002,** *37,* 867–877.
27. Schiller, J.; Süß, R.; Fuchs, B.; Müller, M.; Petkoviæ, M.; Zschörnig, O. Waschipky the Suitability of Different DHB Isomers as Matrices for the MALDI-TOF MS Analysis of Phospholipids: Which Isomer for What Purpose? *Eur. Biophys. J.* **2007,** *36,* 517–527.